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Development of innate immune cells from human pluripotent stem cells

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Mouse and human pluripotent stem cells have been widely used to study the development of the hematopoietic and immune systems. Although not all cells can be derived with the same efficiency, immune cells such as natural killer (NK) cells and macrophages can be easily produced from PSCs to enable development of new cell-based therapies. NK cells and macrophages are part of the innate immune system, the first line of defense against malignancies and infectious disease. Human embryonic stem cell (hESC)- and induced pluripotent stem cell (iPSC)-derived NK cells can be produced at a clinical scale suitable for translation into clinical trials. Additionally, PSCs can be genetically modified to produce hESC/iPSC-derived human NK cells with enhanced antitumor activity. These engineered NK cells can express a stabilized version of the high-affinity Fc receptor CD16, chimeric antigen receptors, or other strategies to enable more potent and targeted cellular immunotherapies. Moreover, macrophages can also be routinely and efficiently produced from hESCs and iPSCs as a tool to expand our knowledge of the basic biology of these cells. hESC- and iPSC-derived macrophages can also be employed as a novel approach for cancer immunotherapy, as well as a strategy to repair or regenerate diseased and damaged tissues and organs.

Mouse and human pluripotent stem cells have been now used for decades in key studies to better understand mammalian hematopoiesis. Studies using mouse embryonic stem cells (ESCs) to create genetic knockout mice have been instrumental in defining key genetic mechanisms that mediate the development of different hematopoietic lineages [1,2]. In an effort to move this work into a human system, our group was the first to derive blood cells (mainly myeloid and erythroid cells) from human ESCs (hESCs) [3] and to produce lymphocytes [4]. These initial studies on hESC-derived lymphocytes produced natural killer (NK) cells with phenotype and function similar to NK cells isolated from peripheral blood, including the ability to kill diverse tumor cells in vitro and in vivo [4,5]. We have subsequently used human induced PSCs (hiPSCs) to produce NK cells and also demonstrated the ability of hESC/hiPSC-derived NK cells to kill virally infected cells, most notably HIV [6,7].

As discussed in this review, these hESC/iPSC-derived NK cells can now be translated into clinical therapies to better treat relapsed and refractory cancers. Additionally, hESCs/iPSCs provide a stable and standardized platform for genetic modification to produce NK cells with improved antitumor activity. In particular, we have produced iPSC-derived NK cells that express chimeric antigen receptors (CARs) to improve killing of typically NK-cell-resistant tumors [8]. Although not covered in this review, other innate lymphoid cells can also be derived from hESCs and hiPSCs [9].

There is obviously considerable interest to also produce cells of the adaptive immune system (T cells and B cells) from hESCs/hiPSCs; however, this has been a much more challenging endeavor and is covered elsewhere in this review series. Although several groups have produced T cells from hESCs [10–12], this is typically markedly less efficient than T-cell development from hematopoietic stem/progenitor cells (HSPCs) isolated from umbilical cord blood (UCB) or NK-cell development from hESCs and iPSCs. Indeed, we previously identified key transcriptional regulators that are differentially expressed in hESC-derived $CD34^+CD45^+$ hematopoietic progenitor cells (HPCs)

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compared with UCB CD34/45$^+$ cells. Specifically, upregulation of *ID* genes in hESC-derived cells may skew lymphocyte development toward NK cells and inhibit T- and B-cell lineages [13]. The production of T cells from iPSCs that were reprogrammed from T cells (termed T-iPSCs) is markedly more efficient than T-cell development from hESCs or non-T-cell-derived iPSCs [14–16]. This finding suggests that epigenetic memory [17] or T-cell receptor rearrangement in T cells alleviates a block in T-cell development. Also notable is the relative paucity of evidence of mature B-cell production from hESCs/hiPSCs, though some systems have been successful at B-cell development [18,19]. Conversely, macrophages are another major effector cell type of innate immunity that play an important role in tissue homeostasis, including recognition and elimination of pathogens and malignant cells. However, macrophage research has been mostly conveyed by using blood monocyte-derived macrophages, which require large amounts of blood from donors (donor-specific variation) and are relatively hard to genetically manipulate. Therefore hESC/iPSC-derived macrophages provide an attractive alternative system for deriving terminally differentiated and consistent human macrophages. This review focuses on the production and potential clinical translation of NK cells and myeloid cells (monocytes) from hESCs and hiPSCs.

NK cells

NK cells were identified in 1975 by two different groups as immune cells able to kill tumor cells without human leukocyte antigen (HLA) restriction and without prior sensitization [20,21]. These properties contrast to T cells that require recognition of self-HLA molecules to mediate their activity. NK cells are recognized to play a central role in the early response to viral infection and tumors and are also involved in organ transplant rejection [22–24]. NK cells are considered innate immune cells because of their early response to infections without prior sensitization and absence of antigen-specific cell surface receptors, although they do express a repertoire of activating and inhibitory receptors that regulate their activity (Figure 1) [25]. NK cells utilize these receptors to recognize key elements on tumor cells and virally infected cells and mediate potent cytolytic activity and cytokine production [26,27]. Because NK cells function as allogeneic effector cells, they do not need to be isolated from individual patients (as with autologous T-cell-based therapies) for cellular immunotherapies.

NK cells originate from CD34$^+$ HSPCs in the bone marrow (BM) and represent 5–15% of circulating lymphocytes in most healthy individuals [28]. Under normal physiological conditions, they are present in a broad range of tissues, including but not limited to the skin, liver, gut, lungs, and kidneys [29,30]. As noted, NK cells express a combination of activating and inhibitory receptors that control cell stimulation and their effector functions. Normal healthy cells express HLA class I molecules on their surface that engage killer immunoglobulin-like receptors (KIRs) that play a key role in self-tolerance of NK cells. In contrast, tumor cells or virally infected cells often downregulate HLA class I expression to escape T-cell cytotoxicity, leading to less inhibitory signaling and increased cytolytic activity by NK cells. Ligands for NK-cell-activating receptors are often upregulated by cellular stress associated with viral infection or tumor development [26,27] and receptor engagement can lead to the

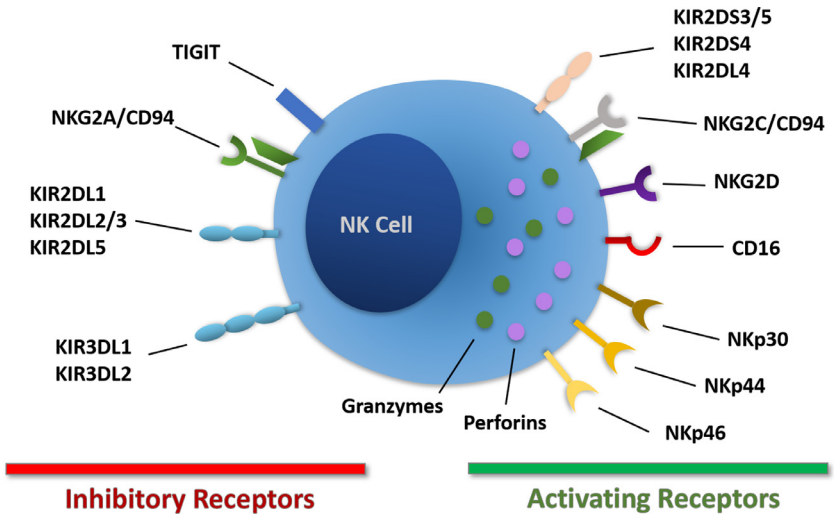

Figure 1. Expression of key NK-cell surface receptors. NK cells express inhibitory and activating receptors that regulate their effector functions.

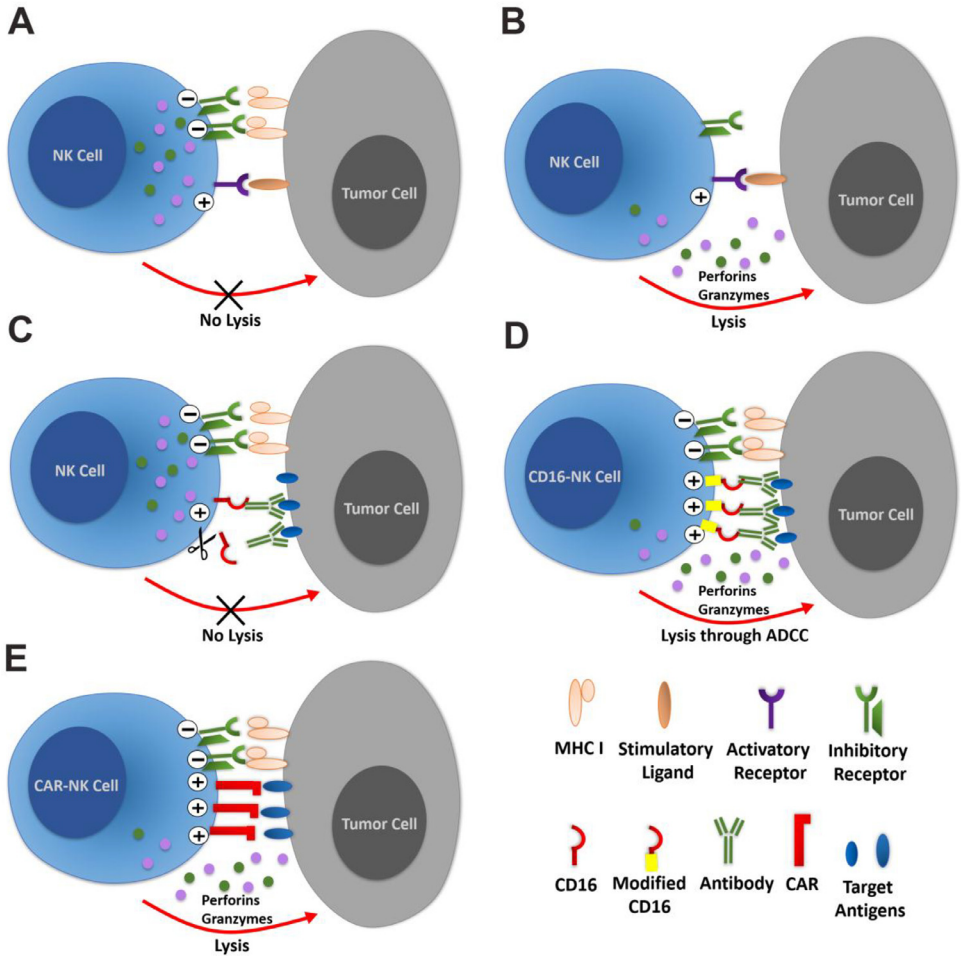

Figure 2. Regulation of NK-cell activity. NK cells are a key meditator of antitumor and antiviral activity. **(A)** Normally, NK-cell activity is balanced by engagement of activating and inhibitory receptors to prevent killing of normal (nontumor and noninfected) cells. **(B)** Tumor cells may downregulate expression of major histocompatibility complex (MHC) class I molecules to avoid T-cell-mediated killing that requires MHC class I expression. MHC class I molecules bind KIRs that mediate inhibitory signals for NK cells. Therefore the loss of MHC class I on tumor cells can shift the balance toward activating receptors, leading to the secretion of granules containing granzymes and perforins to kill the tumor cells. **(C)** Another mechanism of action for NK cells is the recognition of specific antigens through the binding of CD16 with antigen-specific antibodies even if the overexpression of inhibitory ligands by tumor cells and the cleavage of CD16 on NK cells may inhibit their activation. **(D)** Overexpression of modified noncleavable CD16 on hESC- or hiPSC-derived NK cells can increase the activating signal to overcome the inhibitory signals from tumor cells. **(E)** CAR-expressing NK cells, such as those that can be derived from hESCs or hiPSCs, can mediate antitumor activity by providing additional activating signals through the specific binding of the CARs to their antigens expressed on tumor cells.

initiation of NK-cell-mediated cytotoxicity, secretion of proinflammatory cytokines and elimination of target cells. NK-cell-activating receptors include NKG2D, NKp30, NKp44, and NKp46 (Figures 1 and 2). Additionally, most NK cells express the low-affinity activating receptor FcγRIIIa (CD16a), which binds the Fc domain of immunoglobulins to mediate antibody-dependent cellular cytotoxicity (ADCC) [23, 28].

Adoptive NK-cell immunotherapy

The ability of allogeneic NK cells to kill tumor cells has led to many clinical trials for over a decade [31]. Initial studies by Miller et al. demonstrated the efficacy of allogeneic NK cells in treatment of hematological malignancies, most notably finding that 30–50% of patients with refractory or relapsed acute myeloid leukemia could achieve complete remission after receiving allogeneic peripheral blood (PB) NK cells [32–36]. Many clinical trials using NK-cell-based immunotherapy are now recruiting patients for treatment of hematologic or solid tumors [31,37,38]. NK cells isolated from PB have been the most common source for clinical trials involving NK-cell-based immunotherapies [31], but alternative sources include expanded UCB-derived NK cells (UCB-NK) [39–41] and cell lines such as NK-92 [42]. Although NK-92 cells demonstrate antitumor activity and a good safety profile in clinical trials [42], they are aneuploid and therefore need to be

Table 1. In vitro generation of NK cells and macrophages

Cell production	Serum and feeder status	Hematopoietic differentiation protocols	NK cells or macrophages differentiation protocols	Yield	Reference(s)
hESC-NK cells	Feeder-free, serum-free (APEL medium) or with BSA (BPEL)	Spin EB, 8–11 days (BMP4, SCF, VEGF)	EB on coated plates, 28–35 days (IL-15, IL-7, IL-3, FLT3L)	1- to 2-log expansion of the hESC-derived $CD56^+$ $CD45^+$ NK cells; 2- to 3-log expansion using artificial APCs in long-term culture	[45,47]
hESC/hiPSC-NK cells	Serum and feeder-dependent	PSC plated on S17 or M210 stromal cells for 17–21 days	Sorted $CD34^+$ hematopoietic progenitors plated on EL08-1D2 feeder cells, 21–35 days (IL-15, IL-7, IL-3, FLT3L)	1- to 2-log expansion of the hESC-derived $CD56^+$ $CD45^+$ NK cells; 2- to 3-log expansion using artificial APCs in long-term culture	[4,5]
hESC/hiPSC-NK cells	Serum-dependent and partially feeder-dependent	Spin EB, 8–11 days (BMP4, SCF, VEGF)	EB plated on EL08-1D2 feeder cells 28–35 days (IL-15, IL-7, IL-3, FLT3L)	1- to 2-log expansion of the hESC-derived $CD56^+$ $CD45^+$ NK cells; 2- to 3-log expansion using artificial APCs in long-term culture	[88]
hESC/hiPSC-Mφ	Serum-free, feeder-free	Spin EB, 4 days (BMP4, SCF, VEGF)	IL-3, M-CSF (Mφ progenitor cells and M-CSF mature Mφ); first harvest after 21 days and multiple harvests with reducing yields	$\sim 2.5 \times 10^6$ cells over 4 weeks from one well of six-well plate	[68]
hiPSC-Mφ	Serum-free, feeder-dependent	Spin EB, 5 days (mouse feeder layer, bFGF)	IL-3, M-CSF (Mφ progenitor cells), and M-CSF; first harvest after 21 days and multiple harvests with reducing yields	$\sim 5 \times 10^5$ to 1×10^6 cells/week from one well of six-well plate	[89]
hiPSC-Mφ	Serum-free, feeder-free	Spin EB, 6 days (BMP4, SCF, VEGF)	IL-3, M-CSF (Mφ progenitor cells), and M-CSF mature Mφ; 7–10 days for first harvest, multiple harvests with reducing yields	$\sim 4 \times 10^6$ macrophages over 4 weeks from one well of six-well plate	Unpublished data (Kaufman laboratory)

APC=Antigen presenting cell; APEL=albumin polyvinyl alcohol essential lipid; bFGF=basic fibroblast growth factor; BMP4=bone morphogenetic protein 4; BPEL=bovine serum albumin polyvinyl alcohol essential lipid; BSA= bovine serum albumin; VEGF=vascular endothelial growth factor.

irradiated prior to administration. This irradiation prevents expansion of the cells after infusion and limits their survival in patients [43]. Because expansion of NK cells after adoptive transfer clearly correlates to improved activity [33], this need to irradiate NK-92 cells limits their antitumor activity. There are also limitations in using allogeneic PB as the source of NK cells because the yield of cells can be strongly donor dependent and the cell population remains quite heterogeneous, leading to activity that varies from donor to donor [33]. Different NK-cell populations isolated or derived from UCB have also been used in clinical trials to treat hematological malignancies [31,39,40,44]. However, like PB NK cells, UCB-NK cells differ between donors and need to be expanded prior to treatment.

NK cells derived from hPSCs

hESC-derived NK cells were first produced by coculturing hESCs with murine stromal cells (S17 and C166 cells) that had previously demonstrated the ability to sustain expansion of human HSPCs. This coculture led to the production of hESC-derived clusters of $CD34^+CD45^+$ HPCs that were subsequently cultured on a second stromal cell population (AFT024 cells) to obtain $CD45^+CD56^+$ NK cells (Table 1) [4]. These hESC-derived NK cells were able to lyse human tumor cells by both direct cell-mediated cytotoxicity and ADCC while also producing NK-cell-specific cytokines. hESC-NK cells were shown to more effectively mediate tumor (leukemia) clearance in an in vivo mouse xenograft model than UCB-NK cells [5]. Subsequent studies aimed to use more defined conditions for hESC/hiPSC-derived NK cell production, leading to a novel three-step protocol using stromal-free and serum-free conditions [45]. Here, we use a "spin embryoid body" (EB) protocol in serum-free medium to derive $CD34^+CD45^+$ HPCs from hESCs or hiPSCs (Table 1) [45–47]. The EBs are then transferred to gelatin-coated plates and cultured in media containing interleukin-3 (IL-3), IL-15, IL-7, stem cell factor (SCF), and fms-like tyrosine kinase 3 ligand (FLT3L), where they differentiate into both autologous stromal cells and $CD45^+CD56^+$ NK cells. Finally, hESC/hiPSC-derived NK cells can be expanded to clinical scale using a membrane-bound IL-21-expressing cell line [45]. Like PB-NK and UCB-NK cells, hESC/iPSC-derived NK cells express activating and inhibitory receptors such as CD16a, NKp44, NKp46, NKG2D, TRAIL, and KIRs (Figure 3) [45]. Likewise, when cultured under these conditions, hESC/hiPSC-NK cells demonstrate potent cytotoxic activity against both hematologic and solid tumors through both direct cell-mediated activity and ADCC while producing cytokines such as interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) [4,48]. Studies that inject millions of hESC/hiPSC-NK cells into immunodeficient mice have never demonstrated development of teratomas [5,48].

Human PSCs as a resource to produce NK cells with improved antitumor activity

The unique biology of NK cells allows them to serve as a safe and effective allogeneic immunotherapeutic strategy. NK cells can be genetically modified to express CARs or to adapt their receptor repertoire such as through deletion of inhibitory receptors or expression of cytokines (Figure 2). To overcome the reduced efficiency of genetic modification of mature NK cell through transfection [49], hESCs and hiPSCs can be modified in an undifferentiated state utilizing either viral (retroviral or lentiviral vectors) or nonviral transposon systems (*Piggybac* or *Sleeping Beauty*) and then differentiated to produce genetically modified NK cells [8,50].

Initial studies to enhance the activity of hESC/hiPSC-derived NK cells used a CD4/CD3ζ CAR [6,7]. In this study, the CD4/ζ hESC and hiPSC-derived NK cells were able to suppress the replication of HIV in vitro and in vivo and the viral load in the PB of treated mice was significantly reduced. For antitumor CAR-NK cells, instead of using CAR constructs that were originally designed for T-cell-based therapies, subsequent studies used novel NK-cell-specific CARs utilizing NK-cell-specific transmembrane and costimulatory domains. Specifically, after screening 10 different NK-CAR constructs, we demonstrated that iPSCs engineered with a CAR consisting of the transmembrane domain of NKG2D, the 2B4 costimulatory domain and the CD3ζ signaling domain (NKG2D-2B4ζ) mediated strong antigen-specific NK-cell signaling. This NKG2D-2B4ζ CAR (termed CAR4) was linked to a ScFv specific for the tumor antigen mesothelin. When these iPSC-derived CAR4-NK cells were assessed in a xenograft model of ovarian cancer, they showed enhanced antitumor activity compared with a third-generation CAR-T cell expressed in NK cells. Additionally, direct comparison between conventional CAR-T cells and the iPSC-derived CAR4-NK cells demonstrated improved survival and less toxicity from the hiPSC-CAR-NK cell therapy compared with conventional CAR-T cells in an ovarian carcinoma xenograft model [8].

NK cells also express CD16a, an Fc receptor that mediates ADCC, a key mechanism behind the efficacy of monoclonal antibody-based therapies against cancer (e.g., rituximab against non-Hodgkin's lymphoma and trastuzumab against breast cancer [51]). CD16a presents two different polymorphisms, one with a higher affinity for the Fc domain of antibodies and one with a lower affinity [52]. CD16a expression has been

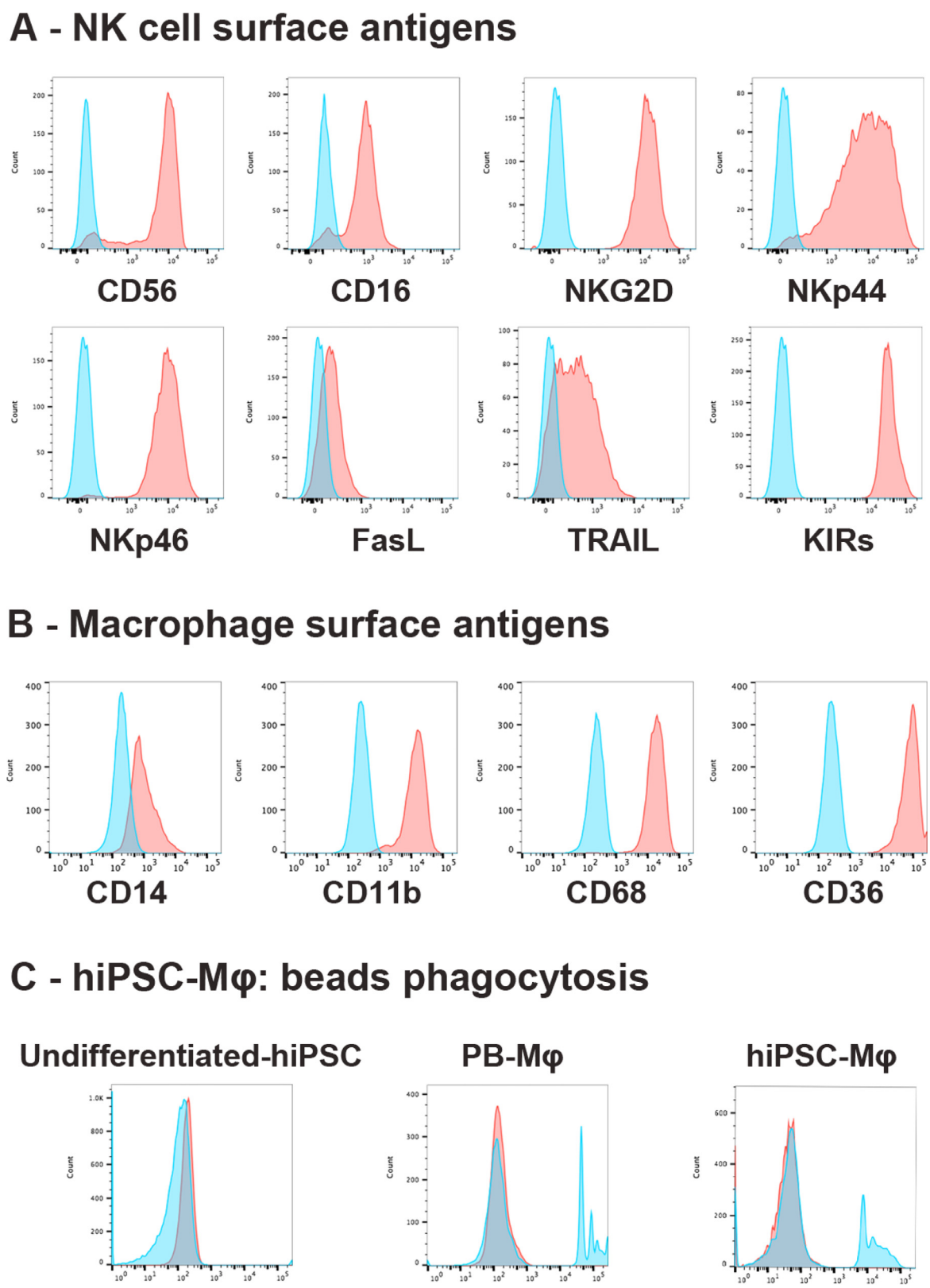

Figure 3. Phenotype and activity of hiPSC-derived NK cells and macrophages. **(A)** Phenotype of hiPSC-derived NK cells and **(B)** phenotype of hiPSC-derived macrophages expressing typical surface antigens. Each of these demonstrates expression of surface antigens typical of NK cells and macrophages isolated from PB. **(C)** Phagocytic activity of hiPSC-Mφ is demonstrated by analysis of undifferentiated hiPSCs (negative control), PB-Mφ, and hiPSCs-Mφ as analyzed by flow cytometry (blue filled: cells treated with 2 μm beads; red filled: untreated control).

shown to decrease on NK cells due to a metalloprotease, ADAM17, which cleaves CD16a from the cell surface after receiving activating signals coming from the same receptor [53]. ADAM17 is expressed by both tumor cells and activated NK cells [54]. ADAM17-mediated cleavage of CD16a reduces their cytolytic activity (Figure 2). To overcome this mechanism, iPSCs were engineered to express a noncleavable version of CD16a and iPSC-NK cells with this CD16a/S197P [54] and demonstrated improved ADCC [53]. These studies illustrate the important ability to be able to routinely genetically modify hESCs/iPSCs, as well as produce clinical-scale quantities of NK cells that now enable these human PSCs to serve as a valuable platform to generate NK cells suitable for allogeneic, "off-the-shelf" cellular immunotherapy against cancers that are refractory to conventional therapy, as well as for possible treatment of chronic viral infections. Phase 1 clinical trials using iPSC-derived NK cells are planned to start in early 2019 to treat relapsed and refractory cancers, the first human PSC-derived blood product to enter the clinical arena.

Use of human pluripotent stem cells to derive therapeutic macrophages

Initial studies to demonstrate hematopoietic differentiation of hESCs produced HPCs that made stereotypic myeloid colonies when plated in semisolid methylcellulose-based medium containing hematopoietic cytokines [3]. Importantly, these hematopoietic colonies demonstrated differentiation of hESCs into myeloid, erythroid, and megakaryocytic cells that were essentially the same as those produced by HPCs isolated from BM or UCB. Sorting for $CD34^+$ cells and quantification of these hematopoietic colony-forming cells further illustrated that hESC-derived hematopoietic cells were similar to "normal" blood cells. Additionally, these studies found that monocytes and macrophages were relatively straightforward to produce from hESCs using the stromal-based differentiation system described previously.

Macrophages are myeloid cells that contribute to numerous functions including tissue development, homeostasis, tissue repair, and innate immunity against invading pathogens and cancer [55]. Distinct types of macrophages develop from different developmental hematopoietic sites: the embryonic yolk sac (YS), the fetal liver (FL), and the postnatal BM [56]. Macrophages that arise from the YS are independent from HSC precursors and populate in the brain as microglia [57]. The FL is populated with YS-derived macrophages and constitutes the major source for other tissue-resident macrophage (TRM) populations, including those found in the liver, lung, spleen, pancreas, and kidney. Those macrophages derived from the YS and the FL reside in the tissues during embryonic development, persist into adulthood, and self-renew during adult life through a Myb-independent proliferation mechanism (independent from HSCs) [58–60].

BM-derived macrophages arise from postnatal HSCs that originate in the aorta-gonad-mesonephros of the embryo and are dependent on the transcription factor c-myb in their development [61,62]. In contrast to YS/FL-derived macrophages, BM-derived macrophages have a short life and populate tissues only under inflammatory conditions [63]. Studies of TRMs involve the isolation of these cells from different tissues with mechanical dissociation techniques [64–66]. Although effective, this strategy is not optimal due to the impact on gene expression patterns upon isolation procedures, possible contamination of RNA from phagocytosis of host tissue cells, and the lack of access to sufficient numbers of appropriate cells. Alternatively, macrophages can be generated in vitro from human PSCs. Numerous studies (Table 1) have utilized mouse ESC and iPSC systems to efficiently produce macrophages in vitro [58,67,68]. In addition to stromal-based methods, EB formation combined with colony-stimulating factor 1 (CSF-1), macrophage-CSF (M-CSF), and IL-3 supplementation has been shown to produce macrophages from hESCs [67]. Additionally, hiPSCs have also been used to generate a homogeneous population of nonadherent monocytes (>90% $CD14^+$) from the culture supernatant. These cells can be continuously harvested for several months while maintaining the ability to differentiate into mature macrophages. These iPSC-derived macrophages demonstrate typical characteristics, including the ability to endocytose lipoprotein, phagocytose opsonized yeast particles, secrete specific cytokines in response to lipopolysaccharide (LPS), and be activated differentially with IFN-γ and IL-4 [68]. More recently, our group has developed a method to rapidly generate large numbers of macrophage progenitor cells and mature macrophages ($\sim 4 \times 10^6$ per well in a six-well plate format over a course of 4 weeks) from hiPSCs using defined feeder and serum-free culture conditions that yield a highly consistent and pure population of macrophages ($CD14^+$, $CD11b^+$, $CD68^+$, and $CD36^+$), which are able to phagocytose latex beads (Figure 3). Despite a similar phenotype and function, hESC/iPSC-derived macrophages have some key differences from adult BM-derived or PB monocyte-derived macrophages, likely related to their development via an Myb-independent mechanism, similar to TRMs. Therefore care should be taken when interpreting the result of studies relying on such methods.

Use of hESC/iPSC-derived macrophages for infectious disease modeling

Recent studies using iPSC-derived macrophages (iPSC-Mφ) have been used to better understand pathogenesis

of Zika virus (ZIKV) and Dengue virus (DENV) infections, two closely related mosquito-transmitted flaviviruses that lead to various clinical outcomes [69]. Specifically, whereas both viruses were able to infect iPSC-Mφ, only DENV and not ZIKA activated secretion of migration inhibitory factor and diminished macrophage migration. Conversely, ZIKA-infected iPSC-Mφ demonstrated enhanced migration and decreased pro-inflammatory responses. These results are consistent with previous observations that, after recognition of pathogen-associated molecular patterns such as double-stranded RNA, macrophages become activated and secrete a broad array of pro-inflammatory cytokines (e.g., IL-1B, TNF-α, and IL-6) and chemokines (e.g., CCL4 and CXCL8/IL-8) that enhance host immune responses [70].

The use of pluripotent stem cell-derived macrophages also enables novel studies on host–pathogen interactions and the role of human genetics in the outcome of infections. This is especially relevant for infectious agents requiring macrophages as a source of persistence and replication, such as *Salmonella* [71], *Chlamydia* [72], and HIV [73], which had remained largely unexplored due to the lack of an appropriate model system. Interestingly, iPSC-Mφ infected with Bacillus Calmette–Guérin resulted in cell apoptosis as well as nitric oxide and TNF-α secretion similar to macrophages generated in vitro from a human monocyte cell line [74]. These studies suggest that PSC-Mφ could be utilized to unravel the distinct impact of infectious agents on disease pathogenesis.

Regenerative-cell-based therapy for macrophage-related diseases

IPSC-Mφ also provide a highly attractive source for cell and gene therapy. Specifically, this approach has led to the development of novel therapeutic targets for diseases involving macrophages such as pulmonary alveolar proteinosis and liver fibrosis. Mucci et al. recently evaluated the airway homing, plasticity, and therapeutic efficacy of iPSC-Mφ in a murine model of hereditary pulmonary alveolar proteinosis. They found that a single administration of $2.5–4 \times 10^6$ iPSC-Mφ resulted in efficient airway homing and conversion to an alveolar macrophage phenotype within 2 months while alleviating disease symptoms, displaying significant plasticity and the therapeutic potential of iPSC-Mφ [75]. The same group applied TALEN-mediated integration of the corrected gene granulocyte-macrophage colony-stimulating factor receptor alpha-chain (CD116) into patient-specific iPSCs and demonstrated functionally corrected macrophages with typical morphology, surface phenotype, phagocytic and secretory activity, as well as functional gene expression, suggesting a novel source of cell-based gene therapy for treatment of genetic diseases [76]. The regenerative capacity of murine iPSC-Mφ for liver fibrosis has been evaluated in vivo. Although murine iPSC-Mφ showed comparable morphology and cell surface markers to BM-derived macrophages, they displayed less phagocytic activity, reduced response to classic stimulators such as LPS and IFN-γ and increased response to IL-4 compared with BM-derived macrophages. Therapeutic application of murine iPSC-Mφ significantly diminished the amount of hepatic fibrosis to 50% of control groups and repopulation of Kupffer cells, highlighting the beneficial effect of in vitro PSC-Mφ to repair a model of liver injury [77].

Cell-based therapy of cancer using macrophages

Distinct macrophage populations reside in different tissues of the body and constitute ~5–20% of the cells in every tissue [78] and contribute to a variety of conditions, including the tumor microenvironment [79]. The role of macrophages in the tumor microenvironment seems to be a double-edged sword, with some studies suggesting they contribute to disease progression [80,81]. However, increasing evidence suggests that targeting of macrophages can be used to contribute to tumor elimination and a subsequent cure [82–84]. Based on these findings, several groups have attempted to use hiPSC-Mφ as a novel cell-based therapy approach for cancer therapy. Koba et al. generated an iPSC-derived myeloid/macrophage cell line (iPS-ML) with enhanced proliferation capacity that grew continuously in an M-CSF-dependent manner. A large supply of cells could be readily obtained using this technology for at least several months. Intraperitoneal administration of iPS-ML cells into SCID mice with pre-established peritoneal gastric cancer resulted in massive accumulation and infiltration into the tumor tissues and dramatically suppressed tumor growth. Furthermore, iPS-ML/IFN-β inhibited the intraperitoneal growth of gastric and pancreatic cancers, suggesting that iPS-ML cells are a promising treatment strategy for disseminated cancers for which no standard treatment is available [85]. The same group has also applied the enhanced proliferation approach to the mouse ESC ES-ML cell line. ES-ML expressing an anti-HER2 mAb reduced the number of cocultured mouse Colon-26 cancer cells expressing HER-2. Injection of ES-ML cells with IFN-γ and LPS into mice inhibited cancer progression in the mouse peritoneal cavity and prolonged survival of the treated animals. Interestingly, transporter associated with antigen processing (TAP)-deficient ES-ML cells conferred a therapeutic benefit to major histocompatibility complex-mismatched allogeneic recipient mice, demonstrating the possible use of TAP-deficient iPSC-myeloid cell lines in cancer therapy [86]. iPSC-Mφ have also been developed for

adoptive cell-based therapy of advanced melanoma to overcome the difficulties relating to application on a broad scale and poor infiltration of immune cells in solid tumors. iPS-ML cells genetically modified to express type I IFN (iPS-ML-IFN) exhibited significant inhibition of metastatic human melanoma in immunodeficient mice, with histological examination confirming the presence of macrophages in the tumor nests. These macrophages expressed M1 polarizing markers, influencing the environment toward inflammatory and antitumor conditions [87]. To achieve successful macrophage-based immunotherapeutic, repetitive administration of large numbers of cells might be necessary. Given the fact that the hiPSC-Mφ platform is able to produce cells on a broad scale, an ample number of cells for cancer therapy can certainly be obtained by this approach.

Overall conclusions about hESC/hiPSC-derived NK cells and macrophages

Human pluripotent stem cell-derived blood cells, including NK cells and macrophages, are valuable tools with which to study immune cell biology, disease pathogenesis, and immune-cell-based therapeutics. hESCs and iPSCs provide a platform, not only to develop clinical grade cell products, but also to better understand the developmental biology of innate immune cells during the differentiation process from HPCs to mature cells. NK cells expressing the repertoire of activating and inhibitory receptors commonly expressed on PB-NK can be differentiated from both hESCs and hiPSCs on a clinical scale when cultured with APCs. ESCs/iPSCs can be easily transfected and used to differentiate NK cells to investigate and modify the function of their receptors. Moreover, specific CARs for anticancer therapies can be engineered into hESC/hiPSC-derived NK cells to generate more potent NK cells. Well-defined protocols facilitate large populations of macrophages derived from hESCs/iPSCs that are highly phagocytic and resemble PB monocyte-derived macrophages ($CD14^+$, $CD11b^+$, $CD68^+$, and $CD36^+$). This ability to produce a large number of homogeneous NK cells and macrophages facilitates their clinical translation for novel "off-the-shelf" cell-based therapies.

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